NOZZLE-LESS ELECTROSPINNING OF CURCUMIN LAODED ALGINATE/PVA BLENDED NANOFIBERS FOR WOUND HEALING

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology

In

Biotechnology

By

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CERTIFICATE

This is to certify that the project report entitled "**NOZZLE LESS ELECTROSPINNING OF CURCUMIN LAODED ALGINATE/PVA BLENDED NANOFIBERS FOR WOUND HEALING**" submitted by Mr. **SAGAR UDASEEN** in partial fulfilment of the requirements for the award of the Masters of Technology in Biotechnology and Medical Engineering with specialization in "Biotechnology" at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance.

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Acknowledgement

"In the name of God, the most merciful and most beneficent"

Firstly I would like to thank the Almighty, as he gave me the serenity to accept the things I cannot change, courage to change the things I can and wisdom to know the differences.

Acknowledgement for a few might be just a trifle thing written on a piece of paper. But in its true essence it gives me an opportunity to remember and express my feelings for those who I love, revere and share secret with. Here I get a chance to express my token of thanks to people who have touched me in a way or the other by their deeds. Words are not enough to express my feelings, which come straight from heart for my college **NIT Rourkela** for providing me exposure to work as an institute of repute.

I wish to express my sincere thanks to my project supervisor **Professor Sirsendu Sekhar Ray,** Assistant Professor, NIT Rourkela for his valuable support and guidance during my project. I hearty thank him for providing me suggestions and help at any point of time besides his expert advice, invaluable suggestions and never ending cooperation during my project period and course of this investigation till preparation of manuscript.

My co-guide **Professor Krishna Pramanik,** Head, Department of Biotechnology and Medical Engineering, NIT Rourkela whom I adhere due to her scientific approaches, mellifluous nature, meticulousness and indefatigable attitude.

I take as a privilege to record my deep sense of reverence and thanks to **Prof. Mukesh Kumar Gupta, Prof. Subhankar Paul, Prof. Bibhukalyan Prasad Nayak, Prof. Amitesh Kumar, Prof. Amit Biswas, Prof. Kunal Pal, Prof. A. Thirugnanam, Prof. Indranil Banerjee and Prof. Nandini Sarkar,** faculties and students of department of Biotechnology and Medical Engineering, NIT Rourkela, for their constant guidance and support all throughout my project period.

Enormous help and guidance which I received from **Faculties, Staff members and students from Department of Chemical Engineering, Department of Ceramic Engineering, Department of Chemistry and Department of Metallurgical & Materials Engineering** NIT Rourkela, cannot be over looked those always stood for me at the critical juncture during the M.Tech. Project Program for providing the support with the departmental facilities.

All the word in the lexicon will be futile and meaningless if I fail to express the obligation from the deep of my heart for my reverend parents, **Shri Santoo Ram Udaseen** and **Shrimati Gopi Udaseen,** who displayed enormous strength, courage and perseverance in helping me to complete my degree. Without their ideal thought, love, inspiration and moral as well as financial support, my dreams would not have been materialized.

I do not afford appreciating the solemn cooperation and liberal assistance received from **Parinita Agrawal, Nimal T. R and Krishan Kumar** and other friends for their unmatched help right from start of course here till the departure from Rourkela. It is indeed a great pleasure to acknowledge the love, affection, solemn cooperation by all my classmates.

SAGAR UDASEEN

Abstract

Nozzle free electrospinning has been found as an advantageous measure over other scaffold fabrication techniques for scaling up the process. Present study was tried to optimize the process parameters to fabricate nanofibrous scaffold using sodium alginate and polyvinyl alcohol (PVA) for tissue engineering applications. PVA and sodium alginate were blended in the ratios of 80:20, 70:30, 60:40, 50:50 and 40:60 for fabrication of scaffold. Curcumin was loaded with the sample containing equal amounts of alginate and PVA. Surface tension, viscosity and conductivity analysis were done to evaluate the material properties. Process optimization was carried out by standardizing the voltage, tip-collector distance and speed of rotation for fiber formation. Nanofibers were characterized by scanning electron microscopy (SEM), Fourier transform infrared (FT-IR), X-ray diffraction (XRD) studies, Differential scanning colorimetery (DSC), Thermogravitometric Analysis (TGA) and Film Burst analysis. Curcumin loaded samples were cross linked with glutaraldehyde and analyzed for their biodegradability, antimicrobial activity and *in vitro* drug release. A reduced level of surface tension and conductivity and increased level of viscosity were observed in the blends with increase ratios of PVA. Standard voltage, collector-tip distance and speed of rotation were optimized as 72V, 12cm and 9.2 rpm respectively. SEM analysis revealed the decrease in fibre diameter with higher volumes of sodium alginate. FTIR and XRD data suggested the interaction mechanism in PVA and sodium alginate due to hydrogen bonding. Intermolecular interactions of PVA with alginate through hydrogen bonding might have improved spinnability of the blended system. The optimized process may be used for the mass production of alginate nanofibers to be applicable in wound healing and tissue engineering.

Keywords: Tissue Engineering, Nozzle free Electrospinning, Scaffold fabrication, Alginate.

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1. Introduction

Nanomaterials have attained broad applications in the fields of tissue engineering for their remarkable functionalities possessing large and active surface areas. Many natural polymers like alginate, cellulose, chitin, and silk have potentials to form crystalline nanofibers with the help of downsize processing techniques. These fibers bound to each other with number of hydrogen bonds and thus support living bodies incorporated. Many synthetic polymers like poly (ε-caprolactone), polylactide, and polyglycolide have been electrospun for tissue scaffolding applications (Liang et. al., 2007). Natural fibers serve numerous advantages in terms of high crystallinity, reproducibility, biodegradability, and biocompatibility when compared with fibers prepared from synthetic polymers by bottom-up processing (Shalumon et. al., 2009). Moreover fibers produced form synthetic polymers using cytotoxic organic solvents during fabrication requires extensive washings of solvents and complete removal of toxic components, before using it as bio-vehicle. Natural polymers, those are readily soluble in water due to their hydrophilic behaviour, have low immunogenicity (Lee et. al., 2009). For fabrication of these nanofibers, electrospinning has emerged out as a simplest and cost effective method. Many natural and synthetic polymers and polymer blends have electrospunned successfully using electrospinning process (Huang et. al., 2003). These electrospun fibers can be found applicable for biomedical applications, like controlled drug delivery, wound dressings, and fabrication of tissue scaffolds. The formed mats from such nanofibers possess high surface areas and desired morphologies, which can support proliferation of seeded cells when targeted as tissue scaffold (Chew et. al., 2006). Availability of numerous polymers and their blending can fulfil to tailor many biological and mechanical properties into the scaffold depending upon the desired applications. Polymer blending utilizes the advantages for improving the physiochemical properties of the polymers

used in the process. Miscibility of the polymeric components is one amongst such important property of a blend which certainly affects morphology, mechanical behaviour, permeability and degradation of formulated blend. Polymeric blends are the mixtures where component polymers are held together with secondary forces like hydrogen bonding, dipole-dipole forces and charge transfer interactions (Varnell et. al., 1981; Varnell et. al., 1983; Woo et. al., 1986).

Natural polymers like sodium alginate, chitosan etc. are generally preferred over synthetic polymers due to biodegradable and biocompatible nature, but mechanical properties and process properties are the challenges where synthetic polymers are chosen. To encounter such challenges, polymeric blends of natural and synthetic polymers are used.

Present study deals with the fabrication of curcumin loaded nanofibrous scaffold from the blends of sodium alginate and PVA with the help of nozzle less electrospinning technology and its characterization. The formulation could be used as a bandage in wound healing with controlled drug delivery.

2. Objectives

Present study carried out under following objectives:

1. To standardize the process parameters of nozzle less electrospinning for fabricating nanofibers using sodium alginate and PVA.

2. To characterize the formulated fibers in order to suffice those with higher amounts of sodium alginate.

3. To incorporate curcumin in the blends of alginate and PVA to formulate a system for wound healing applications.

3. Review of Literature

3. 1 Electrospinning

Electrospinning has emerged out as an applicative measure for nanofibers formation. Large scale production of continuous nanofibers for its applications in medical and tissue engineering has remarkably achieved by the use of electrospinning process by adjusting the required fiber diameter (Li et. al., 2004). Electrospinning set up consists of a nozzle accompanied with the needle, voltage supply, sample holder with spinning facility at one electrode and a collector at another electrode. Electrostatic force pulls the fluid once it has been supplied with the high voltage. Chargeable jet then experience turning stretching due to this contact among outside electric field and jet. Solid fibers fabricate as the result of continuous evaporation of solvent from the sample. Polymer based nanofibers are then collected on a fabric material. Large number of polymers and other ingorganic chemicals has been tried successfully for their ability to electrospun. Electrospun fibers are most versatile system for tissue engineering and wound healing applications because of their ideal and easy way of formulation and fabrication, regulating the desired morphology and diameter.

3.2 Nozzle less Electrospinning

Nozzle less technology, as a modification to other electrospinning technologies has provided advantages by mass scale production of nanofibers by opening commercial opportunities with economic importance (Jirsak et. al., 2005). It provides advantages considering reliability, quality consistency and machine maintenance when compared with the other electrospinning techniques. Needle less electrospinning is evolved as advancement in electrospinning technology aiming production of fibers at industrial large scale with a compact space.

Comparison between nozzle electrospinning and nozzle free electrospinning is illustrated in table 1 (Stanislav, 2011).

Table 1: Comparison between nozzle electrospinning and Nozzle free electrospinning

Variable	**Nozzle electrospinning**	**Nozzle less Electrospinning**
Process	Polymer solution is forced downward with the help of needle.	Polymer solution is evenly distributed over the electrode with the provision of rotation.
Voltage Supply	5-20 kV	30-120 kV
Polymeric concentration	10% of the solution	20% or more of the solution
Taylor cone separation	Distance of needle maintains it.	Self-optimized distances
Hydrostatic Pressure	Varies according to the process	No such criterion
Formulated Fiber diameters	80 nm and higher	80 nm and higher

Electrostatic force is generated due to high voltage in the electrospinning process with which an electrically charged jet of polymer solution is produced. This jet of solution elongates and eventually solvent evaporation takes place to deposit the nanofibers on the collector. Ejaculation of the nanofibers depends upon the potential applied, so nanofibers can be fabricated by controlling applied voltages using specialized high voltage pulsed power supply

(Baba et. al., 2010). Jets are initiated naturally in normal conditions in this technology. Partially immersed polymeric solution with continuous rotation forms a thin layer. There will be formation of conical spikes on the polymeric solution surface. On application of high voltage, spikes concentrate to form taylor cone which finally emerges out in the form of the fibers (Fig. 1). Then the formulated fibers are collected on a fabric mat kept on other electrode.

Fig. 1: nanofiber fabrication through nozzle free electrospinning

Nozzle free electrospinning has eventually increased the market of formulated nanofibers on a mass scale with a better ease in the areas of medical and tissue engineering. Gelatin nanofibers fabricated by nozzle free electrospinning has been successfully tried for enhancing wound healing (Dubsky et. al., 2012).

3.3 Electrospun Fibers

Many natural and synthetic polymers have been tried to obtain fiber based scaffolds. This formulated scaffold could mimic the environment provided by extracellular matrix (ECM) of a given specific tissue by surface modifications. These fibers allow easy adherence to seeded

cells, potent proliferation and differentiation with the growth factors. These nanofbers are highly porous with proper interconnection among their pores, exhibit an increased ratio of surface area to volume, highly flexible, and are capable to show blending with other co-polymers during the process of electrospinning (Chronakis, 2005).

3.4 Alginate: natural polymer

Alginate is a natural heteroglycan formed by the combination (1,4)-b-D-mannuronic acid and (1,3)-a-L-guluronic acid and obtained from a brown sea weed (Cottrell et. al., 1980; Whistler et. al., 1993; Imeson, 1997). Sodium alginate is water soluble, biocompatible, biodegradable polyelectrolyte which has been used as a natural polymer in various applications related to tissue engineering (Alsberg et. al., 2002), drug delivery (Augst et. al., 2002), wound dressing (Hashimoto et. al., 2004) etc. Attempts have been made to electrospun alginate solutions blended with several other polymers in order to fabricate nanofibers as pure alginate is not able to electrospun. Different polymers blended with alginate can be glycerol, polyvinyl alcohol (PVA), polyethylene oxide (PEO) etc. to enhance its mechanical behaviour and electrospinnability (Bhattarai et. al., 2006; Jeong et al., 2010; Lu et. al., 2006; Nie et al., 2008, Nie et. al., 2009; Safi et. al., 2007). These copolymers alleviate the charge repulsion among the chains of alginate and thus improve the flexibility of the alginate chains by forming hydrogen bonds (Caykara et. al., 2005; Nie et al., 2008). Specific intermolecular interactions between blended polymers have resulted desirable properties like reduction in low basic cost, improved processability and high electrospinnability.

Pristine alginate is not able to electrospun. A synthetic polymer Poly vinyl alcohol (PVA) can be used to avoid this problem. 8%, 10% and 12% PVA are tried to blend with sodium alginate to make it electrospinnable (Shahidul et. al., 2010). This can improve mechanical behaviour of alginate.

3.5 Poly (vinyl alcohol) (PVA): synthetic polymer

Poly (vinyl alcohol) (PVA) is polyhydroxy synthetic polymer which provides an easy access to sodium alginate which makes it elecrospinnable by forming an intermolecular hydrogen bonding (Salem,, 2001). PVA is soluble in water and has chemical stability, high chemical resistance and biodegradability. PVA has been replicated in many studies as an additive synthetic copolymer with natural polymers to undergo an easy electrospun nanofiber fabrication processes.

3.6 Curcumin

Increased application of various phytochemicals as drug molecules has become a wide area of research. Curcumin ($C_{21}H_{20}O_6$) is a polyphenol phytochemical (fig. 2) which is a present in the extracts of *Curcuma longa.* It is a well known colouring agent used in various food and drug industries (Okada et al., 2001; Joe et al., 2004). Consumption of a dose of 100mg/day curcumin as a dietary source of spice is permissible (Ammon et. al., 1991). Curcumin is a well known drug for its enormous pharmacological activities like antioxidant action, anti-inflammatory agent (Liang et. al., 2008; Williams's et. al., 2007), anti-tumour agent (Maheshwari et. al., 2006), and anti-diabetic action (Aggarwal et. al., 2009). Mode of action of curcumin for preventing inflammation works on the basis of inhibiting enzymes like cyclooxygenase 2 (COX-2) and lipoxygenase (LOX) (Abe et. al., 1999; Huang et. al., 1991).

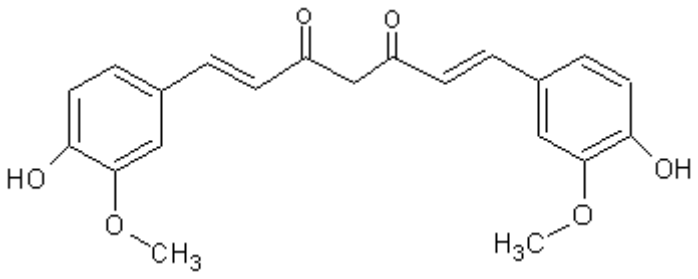

Fig. 2: Molecular structure of curcumin

Panchatcharam et. al. reported the potentials of curcumin for wound healing in rats by slowing down the lipid peroxides expression, and increased expression of superoxide dismutase, glutathione peroxidas and catalase (Panchatcharam et. al., 2006). Inclusion of curcumin in various formulations like liposome, hydrogel, nanofibers and other systems has gained enough advantages in the fields of tissue engineering and wound healing. Liposomes with curcumin have been successfully tried for their antimelanoma activity (Yan et. al., 2012) Poly(ε-Caprolactone) and curcumin based nanofibers showed enhanced rate of wound healing in a diabetic mouse (Jonathan et. al., 2009).

3.7 Electrospun Fibers: Applications

Nanofibers have been successfully tried for their applications in bioengineering, clothing, electronics and microfluidic devices (Vonch et. al., 2007). Nanofibers have been successfully tried for their applications in tissue culture studies (Li et al., 2002; Yoshimoto et al., 2003; Suwantong et al., 2007; Wutticharoenmongkol et al., 2007), drug delivery methods (Kenawy et al., 2002; Taepaiboon et al., 2007; Tungprapa et al., 2007; Suwantong et al., 2007; Suwantong et al., 2008) and as dressing materials in wounds (Noh et al., 2006; Han et al., 2007; Zhou et al., 2008).

Electrospun fibers can be used in many industrial applications considering filtration process (Gopal et. al., 2006), catalyst and enzyme carriers (Kedem et. al., 2005; Jia et. al., 2002), sensors (Wang et. al., 2002), recovery of metal ions (Saeed et. al., 2008; Fang et.al., 2008; Lu et. al., 2009) and affinity membranes (Ma et. al., 2005). Three dimensional nanofibrous structures can be produced by the process of electrospinning which can be applicable as biomaterial scaffolds to support the growth of the cell, wound healing or to deliver desirable drug. Polyionic complexes formed with the help of electrospinning do not require additional crosslinking steps; those can alter the morphology of fabricated fibers. Elecrospun nanofibers

can mimic the structures of native extracellular matrix (LeDuc et. al., 2007). Surface area to volume ratio of electrospun polymeric fibrous scaffolds is high, which can be advantageous for increase in cell to material interactions (Chew et. al., 2006).

Nano-fibrous scaffold formulated using electrospinning is an artificial mode for cell attachment and proliferation by maintaining the phonotypical behaviour of seeded cells. Some uses of nanofibers are showed in the given figure (Fig. 3)

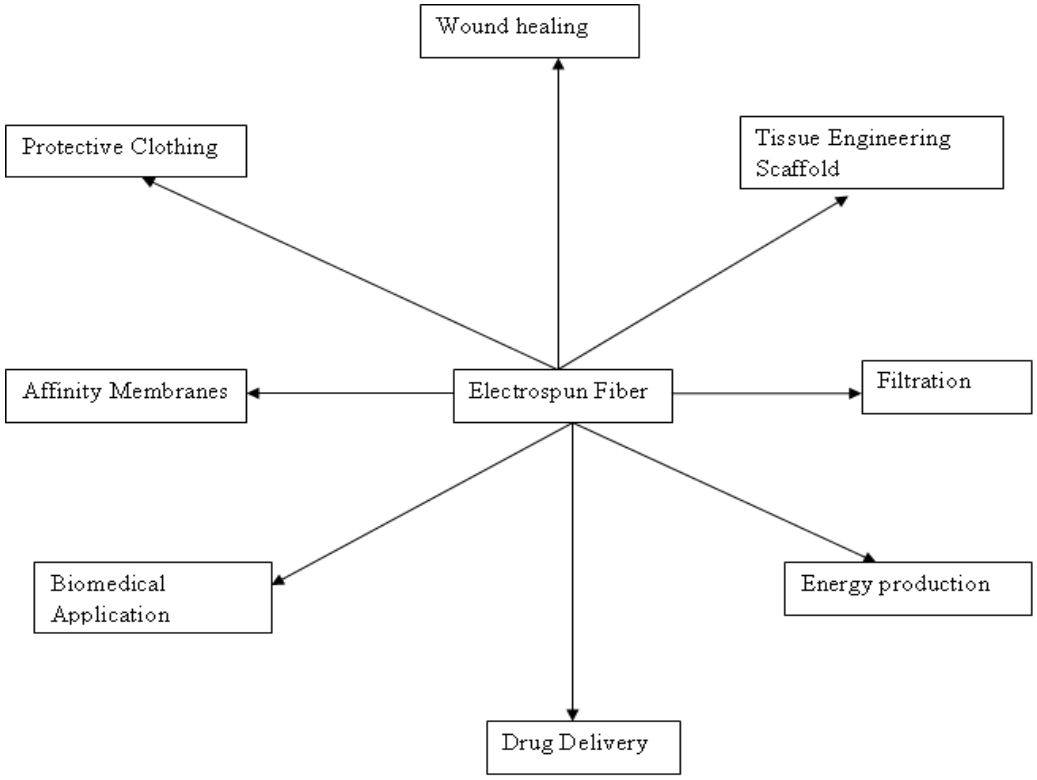

Fig. 3. Applications of electrospun nanofibers in various fields.

3.8 Nanofibers in wound healing

Drug delivery using biomaterial polymeric design is a suitable targeted and effective mode of applications in therapeutic science. Small sizes of the pores, high surface are to volume ratio and high porosity are some of the advantages where nanofibers are routinely used for administration of drugs. The localized area can be targeted with the phenomenon of controlled drug delivery (Gombotz et al., 1995). Drug delivery using nanofibers can be used

to direction growth factors to the locality of application. Repair and regeneration using such bio-polymeric nanofibers are advantageous enough considering drug delivery system and tissue engineered scaffold. Loaded drug can be targeted efficiently with the provision of nanofibers as those serve increased surface area to volume ratio. The principle of drug delivery is basically based upon the increase in dissolution rate with an increase in surface area of drug.

Healing the scars requires moist conditions of environment which is feasibly provided by various heal curing products present in market. Hydrogels; one amongst such marketed products, prevent the further enlargement of necrotic abrasion through apoptosis by providing humid environmental condition (Tarun et. al., 2011). Sodium alginate and poly (vinyl alcohol) (PVA) based hydrogels have been successfully imparted for wound healing applications in rats (Barnett et. al., 1987). Alginate in accounts gets removed from the body after proper dissolution processes (Barnett et. al., 1987). Some drug loaded electrospun polymers in wound healing applications are illustrated in table 2.

Chitosan and poly vinyl alcohol based nanofiber mats were showed as an ideal wound healing material with its biodegradable and antibacterial properties (Natthan et. al., 2012). Nanofibers for their wide functions in improved healing, quick absorption, relieving pain and controlling infection, have remarkably attained the market growth. Verreck et al. have suitably introduced the drugs into nanofiber based scaffold as a dug carrier (Verreck et. al., 2003). Electrospinning of poly-D-lactide (PDLA) was successfully tried to deliver 90% of Mefoxin drug (Zong et al., 2002). Rapid diffusion of biodegradation was seen in such fibers. Zong et al., had showed the complete degradation of Mefoxin in 48 hours of implanting (Zong et al., 2002).

Table 2: Various polymers loaded with different drugs for wound healing applications

S. No.	Polymer Used	Drug Loaded	Applications	Reference
1.	Poly(ε-Caprolactone) and poly(nonamethylene azelate)	Triclosan	Wound healing antimicrobial activity	del Valle et. al., 2012
2.	Poly(ε-Caprolactone)	Eggshell Membrane and Catechin	Wound dressing applications	Kang et. al., 2012
3.	Cellulose acetate	Asiaticoside and curcumin	Removal of transdermal patches and wound dressings	Suwantong et. al., 2010
4.	Chitosan and Poly(ε-Caprolactone)	Rhodamine B and naproxen	tissue engineering, and wound healing	Wang et. al., 2010
5.	Poly(lactic acid), poly(ethylene-co-vinyl acetate) and blend	Tetracycline hydrochloride	Wound healing	Kenawy et. al., 2010
6.	Poly(ε-Caprolactone)	Curcumin	Diabetic Wound Dressing with Antioxidant and Anti-inflammatory	Jonathan et. al., 2009
7.	Cellulose acetate	Asiaticoside	wound dressing patches	Suwantong et. al., 2008
8.	Cellulose acetate	Retinoic acid and alpha-tocopherol	Therapeutic role in transdermal and dermal applications.	Taepaiboon et. al., 2007
9.	Cellulose acetate	Naproxen, indomethacin, ibuprofen and sulindac	Helpful in pain and inflammations	Tungprapa et. al., 2007
10.	Poly(ε-caprolactone)	Dexamethasone	Anti-inflammatory applications in wound healing	Nottelet et. al., 2007

Uniform nanofiber with small diameters were formulated using triethylbenzyl ammonium chloride and sodium dodecyl sulfate in the preparation of Polylactic acid (PLLA) to deliver rifampin (Zeng et al., 2003). Cellular acetate mats containing 5-20% of curcumin was successfully electrospun and fibers in the range of 314-340 nm were formulated. Total immersion method was adopted to release curcumin from such fibers and it showed a release of 90-95% curcumin release (Suwantong et. al., 2007).

4. Materials & Methods

4.1 Materials

Sodium alginate having viscosity of 2,000 cps was purchased from SDFCL, Mumbai, India. PVA (molecular weight: 14,000) was purchased from Otto Chemie Pvt. Ltd., Mumbai, India. All preparations were done in double distilled water (DDW). Curcumin was purchased from MP Biomedicals, India while the cross linking agent Glutaraldehyde solution (25%) was purchased from Merck Pvt. Ltd., India. Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Antibiotic antimycotic solution and Phosphate Buffer Saline (PBS) were purchased from HiMedia, India.

4.2 Preparation of polymeric blend solutions and analysis of material properties

2% sodium alginate was dissolved in DDW at room temperature. 10% PVA was prepared in DDW at 70°C with continuous stirring for 4 hours. Polymeric blends were prepared by mixing PVA and sodium alginate in five different ratios. These volume ratios of PVA to sodium alginate were prepared ranging 80/20, 70/30, 60/40, 50/50 and 40/60. Blended solutions were continuously stirred for 1 hour before use. Surface tension analysis was done to evaluate the material properties by Whilmey plate method using a surface tensiometer, Data Physics, Germany (DCAT-11EC). Viscosity of solutions was tested by Bohlin Visco 88 viscometer (Malvern Instruments, U.K.). Moore Model was applied for the analysis. Conductivity analysis was performed using Deluxe conductivity meter 601, EI Products, India.

4.3 Standardization for electrospinning of blend solutions

Process optimization was done in order to finalize the voltage, tip-collector distance and rotation speed for different blend solutions in order to standardize the electrospinning process

parameters. Different applied voltages were tried at different collector-tip distances for the formation of cones and hence the nanofibers. Sole 2% alginate was also tried for electrospinning at variable parameters.

4.4 Nanofiber fabrication

Nozzle free electrospinning (Elmarco, NS Lab 200) was used to fabricate nanofibers from the blended solutions of sodium alginate and PVA. During the electrospinning process, voltage applied was 72 kV. The tip to collector distance was maintained at 12 cm and a rotation speed of 9.2 rpm was supplied. Fibers were collected on a mat.

4.5 Scanning Electron Microscopy (SEM)

Fibers formed from blended solutions of PVA and sodium alginate in the ratios of 80:20, 70/30, 60/40, 50/50 and 40/60 were analyzed for their morphologies using scanning electron microscope (JEOL-JSM 6480 LV SEM) after coating with a thin layer of platinum. SEM images were used to measure the fiber diameters in order to know the effect of incorporation of one polymer into another and hence the effect of it in fiber formations.

4.6 X-Ray diffraction (XRD)

Blended polymeric solutions in different ratios were analyzed for XRD analysis using X-ray diffractometer (XRD-PANalytical). Samples were scanned for full XRD patterns from 5.00°C - 30.00°C 2θ with step size 2°/min at 30kV tension and 20mA current.

4.7 Fourier-transform infrared (FTIR) spectroscopy

All five blended nanofibers and pristine 10% PVA fibers were examined for spectroscopic analysis using FTIR spectroscopy. Nanofibrous thin sheets were pressed under potassium bromide (KBr) layers. With the help of pressing machine (KBr press Technosearch), a pressure of 10 tons was applied. These sample layers coated with KBr were placed in

machine (IR Prestige-21) to record FTIR readings. Scanning in the range of 4000 cm^{-1} to 500 cm^{-1} was done.

4.8 Thermogravitometric Analysis (TGA)

Thermogravitometric analysis (TGA) and Differential scanning colorimetery (DSC) were used to study the thermal behaviour of formulated fibers. 5 mg of nanofiber was used to study thermogravitometric behaviour using TG-DSC apparatus (STA449C/4/MFC/G apparatus), Netzsch, Germany, under N_2 atmosphere using alumina as reference. Readings were taken out at a temperature range of 25ºC-1000 ºC at a step size of 10ºC/min.

4.9 Differential scanning colorimetery (DSC)

15 mg of samples were used for checking the thermal profiles of fabricated fibers using DSC-200–F3, MAIA, Netzsch, Germany. Samples were taken into aluminum hermetic crucibles and sealed. Samples were analyzed for the temperature range of 20ºC - 300ºC at a heating rate of 10º C/min

4.10 Film Burst analysis

Mechanical texture property (tin film burst assay) was carried out using mechanical tester Stable Microsystems, TA-HD plus, U.K. Fiber films of almost equal sizes (1*1 cm^2) were cut and allowed to undergo the bursting at various loads and thus mean bursting strengths for different films were calculated.

4.11 Biodegradability Assay

Nanofibers mats were cut in almost equal sizes (1*1 cm^2) and weighed. Mats were then incubated in 5 ml of PBS at room temperature (RT). Weights of mats were analyzed after every half an hour and fresh PBS was added in incubated at same conditions. Experiment was

performed in triplicates. Initial weight (W_i) and final weight (W_f) were recorded and percentage of biodegradability was calculated using the given formula:

% Biodegradability = (Wi-Wf)/Wi * 100

4. 12 Preparation of curcumin based polymeric blend solutions

Sodium alginate and PVA were chosen for fabrication of nanofibers. 2% sodium alginate and 10% PVA were separately prepared in double distilled water (DDW). These polymers were mixed in equal amounts and (50:50) and stirred for one hour. 5% Curcumin was loaded in the blended polymeric solution and stirred for a continuous and uniform mixing.

4.13 Nanofiber fabrication

Curcumin loaded blended solution was electrospun to formulate nanofibers using nozzle less electrospinning equipment (Elmarco, NS Lab 200). A voltage of 70 kV with tip-collector distance of 12 cm and 9.2 rpm of rotation speed were maintained for fiber fabrication.

4.14 Characterization of drug based nanofibers

Polymeric fiber mats with and without drug were observed under scanning electron microscope (JEOL-JSM 6480 LV SEM) for their morphological structure with platinum coating. X-ray diffraction of the formulated fibers was analyzed to understand the interaction mechanism of polymers and drug using X-ray diffractometer (XRD-PANalytical). The XRD pattern was executed at the voltage and current of 30kV and 20mA respectively. 2θ range of 5.00°C - 50.00°C at a step size of 2°/min was maintained. Spectroscopic analysis of fibers was done with the help of Fourier-transform infrared (FTIR) spectroscopy. Nanofibers pressed with potassium bromide using KBr press Technosearch instrument were analyzed from 4000 cm^{-1} to 500 cm^{-1} under FTIR machine (IR Prestige-21). Thermal performances of

the fibers were analyzed using Differential scanning colorimetery (DSC) and Thermogravitometric analysis (TGA). For DSC, 15 mg of samples were sealed into aluminum crucibles and analyzed using DSC machine (DSC- 200–F3, MAIA, Netzsch, Germany) at 20ºC -300ºC temperature range with 10º C/min heating range. For, TG analysis, 5 mg of samples were analyzed uging TG-DSC apparatus (STA449C/4/MFC/G apparatus Netzsch, Germany) at 25ºC-600ºC temperature range with 10ºC/min step size.

4.15 Cross linking of Fibers

Air dried fibers loaded with curcumin and without curcumin were kept in a dessicator with 10 ml of 25% glutaraldehyde solution in a petri dish for 2 hours.

4.16 Biodegradability Assay

Nanofibers mats were cut in almost equal sizes and weighed. Mats were then incubated in 5 ml of PBS (pH 7.4) at room temperature (RT). Weights of mats were analyzed after every day and fresh PBS was added in incubated at same conditions. Experiment was performed in triplicates. Initial weight (W_i) and final weight (W_f) were recorded and percentage of biodegradability was calculated using the given formula:

% Biodegradability or degradation index = [(Wi-Wf)/Wi] * 100

4.17 Antimicrobial Activity

Antimicrobial activity was tested on the fiber matrix containing curcumin using *Bacillus subtilis* (MTCC 121) culture. Nutrient agar media was prepared and 1ml of bacterial cell suspension culture with the concentration of 10^{-6} to10^{-7} cfu/ml was spread over the media. Nanofiber matrix with the drug and a control sample (fiber matrix without drug) were applied on the media with bacterial culture. Zone of inhibition was checked after 24 hours of incubation.

4.18 Release of curcumin *in vitro*

Fibers of sodium alginate and PVA with and without curcumin were cut into the matrix of 1*1 cm^2 and incubated in DMEM containing 10% FBS and 1% antibiotic solution at 37°C for 3 days. After every 12 hours, media with released drug were collected and fresh media was added to the fiber matrixes. The collected media was kept at 80°C to allow it to evaporate. Dried samples were mixed with 50% ethanol and the drug released was observed under fluorescence spectrophotometer (Jobin Yvon Fluoromax-4 Spectrofluorometer, Horiba Scientific, Germany) at the excitation of 430 nm.

5. Results and Discussion

5.1 Nanofibers fabrication by electrospinning from blend solutions

Different blend samples in various ratios (2% sodium alginate and 10 % PVA) were electrospun at variable voltages and tip-collector distances in order to optimize the process. Each sample was analyzed at a voltage range of 20-80 kV. Different tip to collector distances from 10-19cm were tried at these variable voltages. It was observed that cone formation in different solutions was started at around 45kV. Uniform fiber formation for different polymeric blend solutions were observed at an applied voltage of 72-77 kV, with tip to collector distance of 12 cm and a rotation speed of 9.2 rpm (Table 3). All five blended polymeric solutions (80:20, 70:30, 60:40, 50:50 and 40:60) and sole 10% PVA were observed to deposit uniform fibers at these ranges. Sole 2% alginate was also tried for its ability of electrospun at various voltage and distance parameters. It was found that sole 2% sodium alginate was not able to fabricate fibers at any of these mentioned parameter ranges. Though there was cone formation for sole 2% alginate at 50 kV, and increase in voltage continued the cone formation and sparking at 80 kV.

5.2 Material Properties Analysis

Surface tension, viscosity and conductivity were analysed from blended polymeric solutions, it was observed that there is a reduction in all the values as the amounts of PVA is increased while viscosity was found to increase with increasing amounts of PVA (Table 4). The data for surface tension and viscosity could help in extracting the information about the parameters in standardizing the process of electrospinning.

Table 3: Results for tip to collector distance and voltage standardization for electrospinning

S. No.	Polymer Solution	Distance (cm)	Voltage (kV)	Comments
A.	2% Alginate	11-19	35-80	No fibres; Cone formation at 50 kV; Sparking at 80 kV
B.	10%PVA+2 % Alginate			
1	80:20	11-19	35-80	Cone Formation at 35 kV; Fibre formation at 72 kV and 12 cm
2.	70:30	11-19	35-80	Cone Formation at 35 kV; Fibre formation at 76 kV and 12 cm
3.	60:40	11-19	35-80	Cone Formation at 35 kV; Fibre formation at 75 kV and 12 cm
4.	50:50	11-19	35-80	Cone Formation at 35 kV; Fibre formation at 72 kV and 12 cm
5.	40:60	11-19	35-80	Cone Formation at 35 kV; Fibre formation at 72 kV and 12 cm

Table 4: Physical and chemical parameters for different polymeric blend solutions

S.No.	Concentration (PVA: Sodium Alginate)	Surface Tension (mN/m)	Viscosity (millisiemens)	Conductivity (µS/cm)
1	100:0	42.214 ± 0.030	4.159 ± 0.05	0.4527±0.005
2	80:20	42.424 ± 0.029	3.567 ± 0.04	0.86±0.047
3	70:30	42.560 ± 0.029	3.396 ± 0.05	0.896±0.003
4.	60:40	42.670 ± 0.030	3.087 ± 0.07	1.0437±0.043
5.	50:50	42.810 ± 0.030	2.871 ± 0.043	1.340±0.016
6.	40:60	42.980 ± 0.030	1.599 ± 0.05	1.634±0.043
7.	0:100	69.693 ± 0.029	2.888 ± 0.05	3.42±0.009

5.3 Scanning Electron Microscopy (SEM)

Morphology of electrospun fibers were examined by their SEM images. SEM images are shown in the fig.4. Uniform fibers were formed at different blended ratios. Decreases in diameters were observed in the blended polymeric fibers as the amount of 2% sodium alginate was increased in to the blended solutions. Fewer beads were found in the blend of PVA and sodium alginate at the ratio of 60:40 (Fig. 4e).

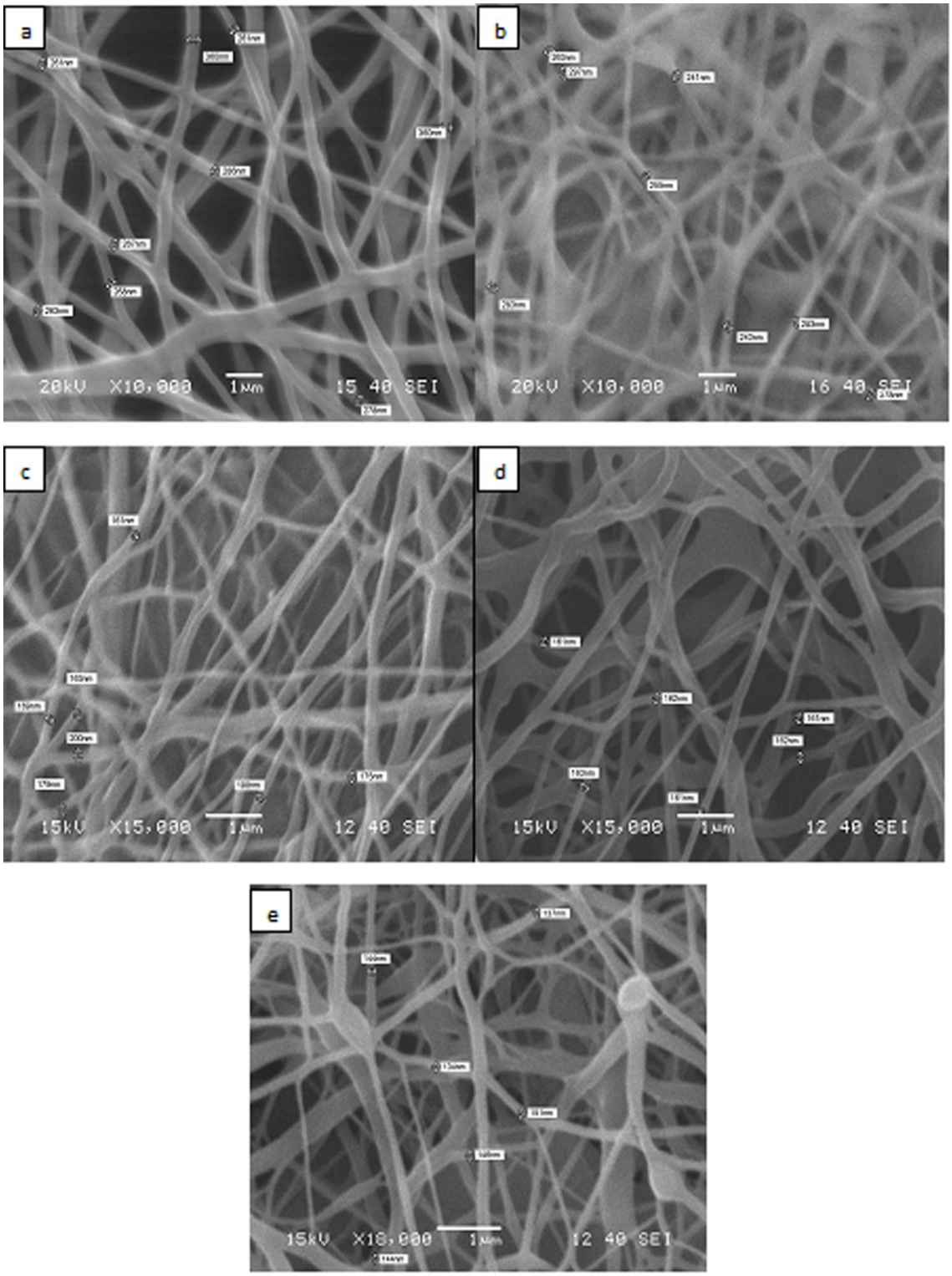

Fig. 4: SEM images of electrospun 10% PVA and 2% sodium alginate blend nanofibers in the ratios of (a) 80:20, (b) 70:30, (c) 60:40, (d) 50:50, and (e) 40:60

Different points were taken to estimate the diameters of fibers and thus average diameter for different formulated fibers were measured. Fibers with the diameter of 400-100 nm were obtained. Average diameters for different five samples are shown in fig. 5.

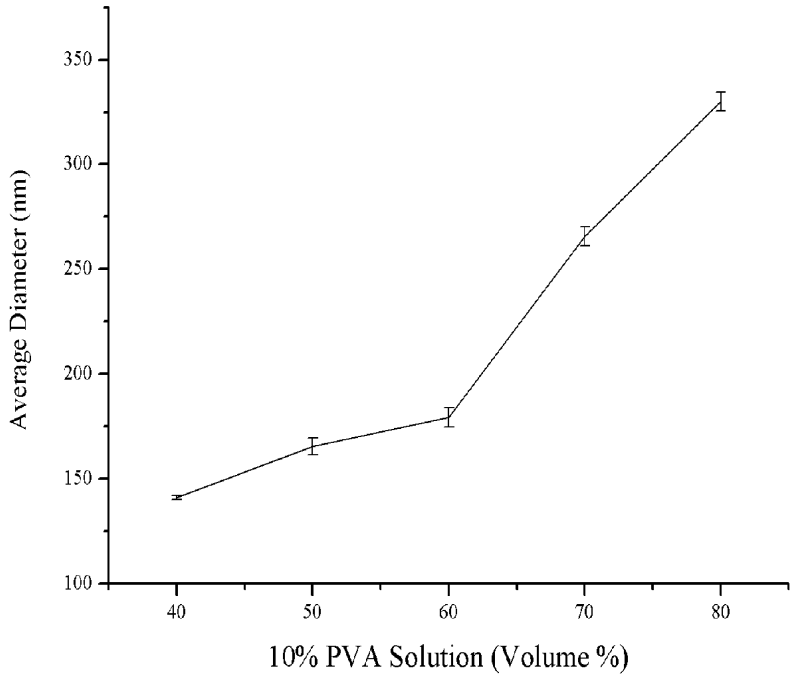

Fig. 5: Average diameters of electrospun nanofibers with 10% PVA and 2% sodium alginate.

5.4 X-Ray diffraction (XRD)

Fig.6 describes the XRD patterns of PVA and sodium alginate blend polymeric fibers. There is strong intramolecular and intermolecular hydrogen bonding in PVA due to which it shows a significant crystalline peak at an angle of about 19.3º (Shahidul et. al., 2010). As the volume of sodium alginate is increased in the corresponding blends, the peak of 19.3º became broader possible due to reduction in crystallinity of the solutions. The reduction of crystallinity is due to the intermolecular hydrogen bonding between carboxyl or hydroxyl groups of sodium alginate with hydroxyl groups of PVA.

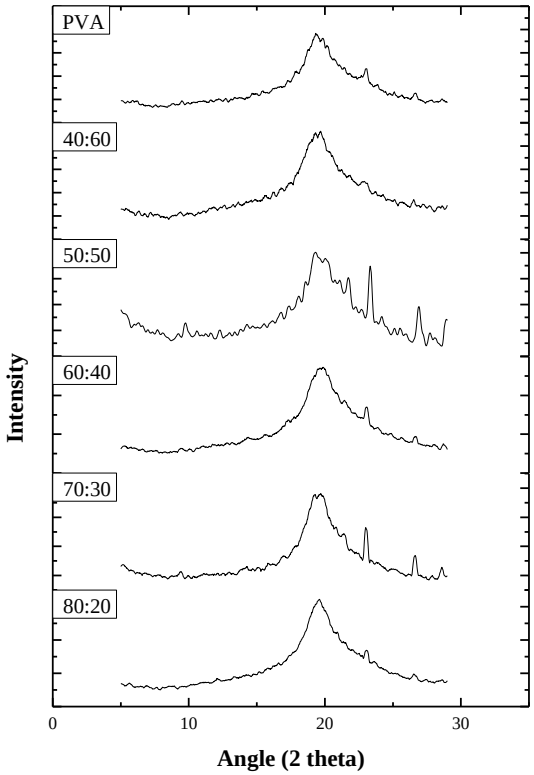

Fig. 6: XRD analysis data for electrospun nanofibers with 10% PVA and 2% sodium alginate in different ratios.

5.5 Fourier-transform infrared (FTIR) spectroscopy

PVA shows various peaks for the presence of different functional groups like CH_2 group at 2944 cm^{-1}, C-O group at 1096 cm^{-1} and hydroxyl group at 3435 cm^{-1} and (Shahidul et. al., 2010). Sodium alginate shows a peak at 3430 cm^{-1} for hydroxyl groups. Asymmetric and symmetric $–COO^-$ group peaks correspond to wavenumber of 1615 cm^{-1} and 1417 cm^{-1} respectively. For alginate, peaks were observed at 3430, 1615 and 1417 cm^{-1}. Fig.7 shows the FTIR spectra of different polymeric blend nanofibers with pristine PVA. It was also observed, that hydroxyl peaks were became broad as the quantity of alginate increased. It

could be because of the formation of hydrogen bonding between hydroxyl groups of PVA and sodium alginate. This phenomenon possible favours the elecrospinnabilty of alginate.

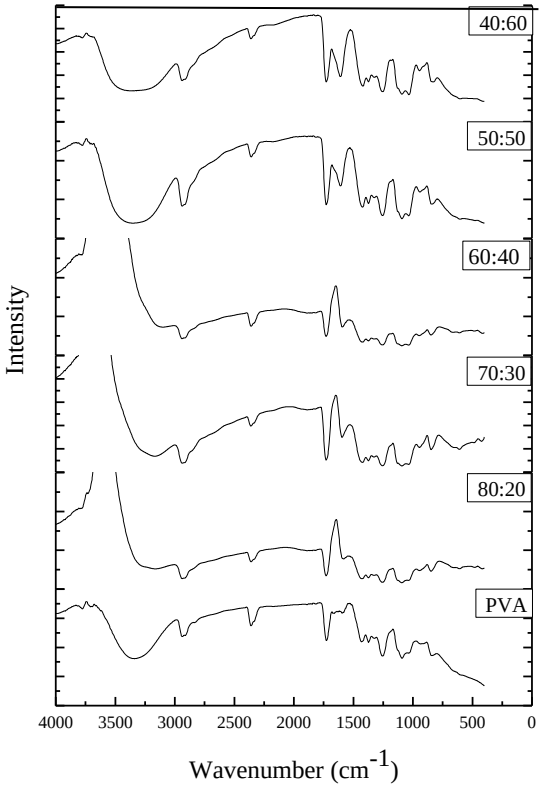

Fig.7: FTIR analysis data for electrospun nanofibers with 10% PVA and 2% sodium alginate in the different blended ratios

5.6 Thermogravitometric Analysis (TGA)

PVA undergoes dehydration and depolymerisation when subjected to pyrolysis at temperatures more than 200ºC and 400ºC respectively (Shahidul et. al. 2010). Fig. 8 shows the TGA thermograph of PVA and blended solutions with sodium alginate. PVA forms a conjugated polyene structure at 245 ºC with elimination of water molecules (Tsuchiya et. al., 1969). The peak at 230-260ºC was observed which could be because of thermal degradation of PVA. This degradation can be followed by vinyl ester formations with the production of

alkene and aldehyde end groups. With an increase of alginate amount, the degradation temperature was increased.

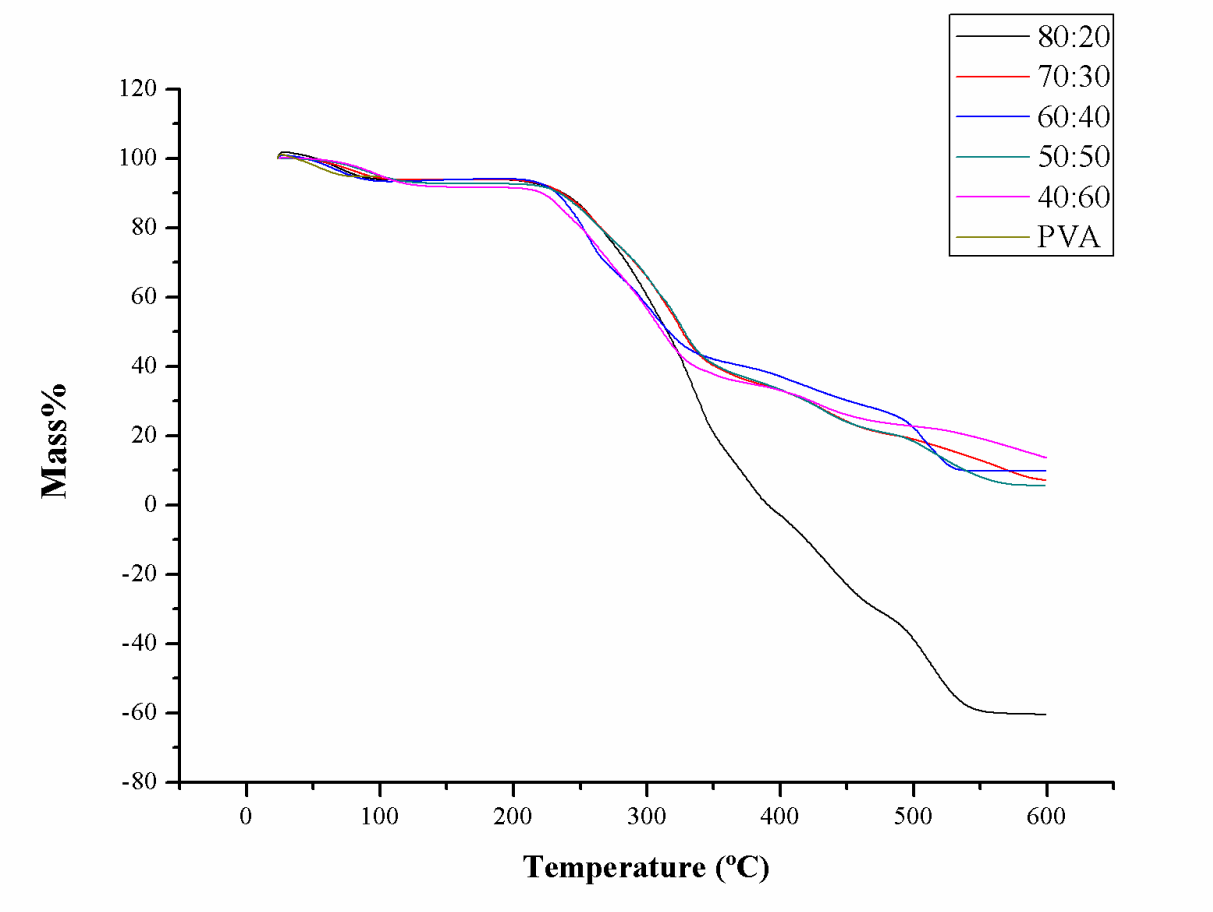

Fig. 8: Thermogravitometric analysis data for electrospun PVA and alginate blend solutions.

5.7 Differential scanning colorimetery (DSC)

Sole PVA and its blend solutions with sodium alginate were analyzed for DSC analysis (Fig. 9). At around 190°C, an endothermic peak has been observed which symbolizes the melting temperature of PVA. On addition of increasing amounts of alginate, this peak has been shown to shift at lower temperature than that of pure PVA.

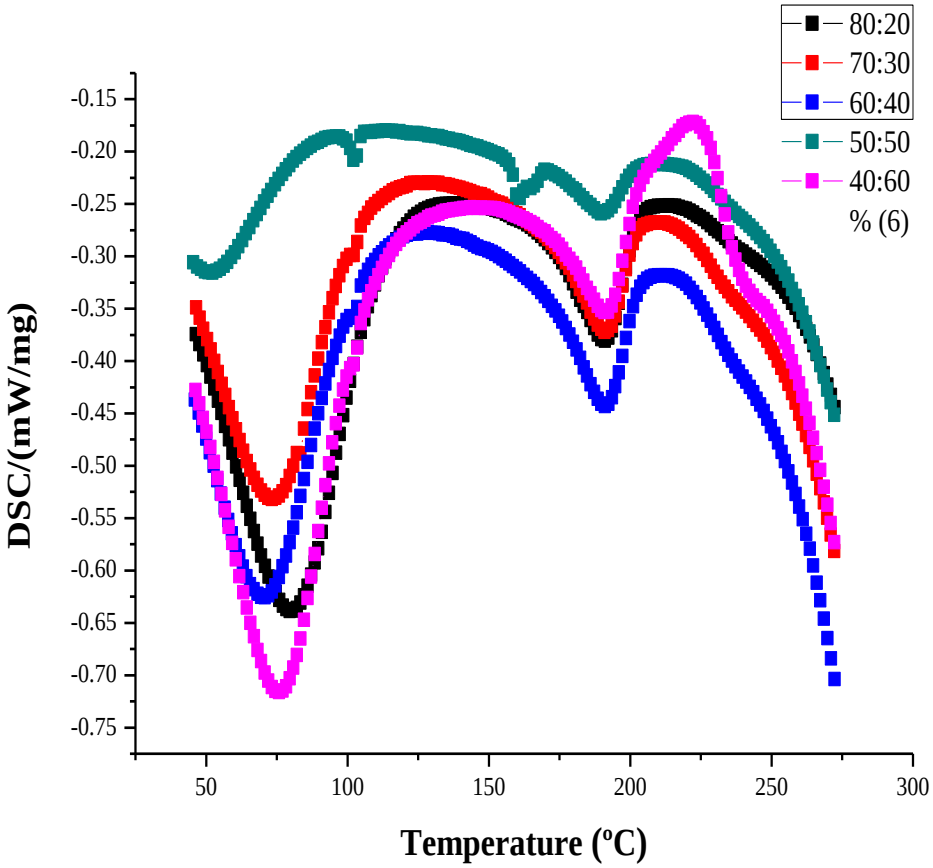

Fig. 9: Differential scanning colorimetery analysis data for electrospun PVA and alginate blend solutions

5.8 Biodegradability Assay

Biodegradability percentage was calculated by incubating the samples in PBS at RT. Thin film prepared from pristine PVA showed complete degradation in 1 hour. While it was observed an increase in the biodegradability % with the accumulation of sodium alginate in PVA making the nanofiber sustainable for enough time in body fluid, once incorporated at the destined place. All blended fibers sustained for 150 minutes and after which complete degradation was seen (Fig. 10).

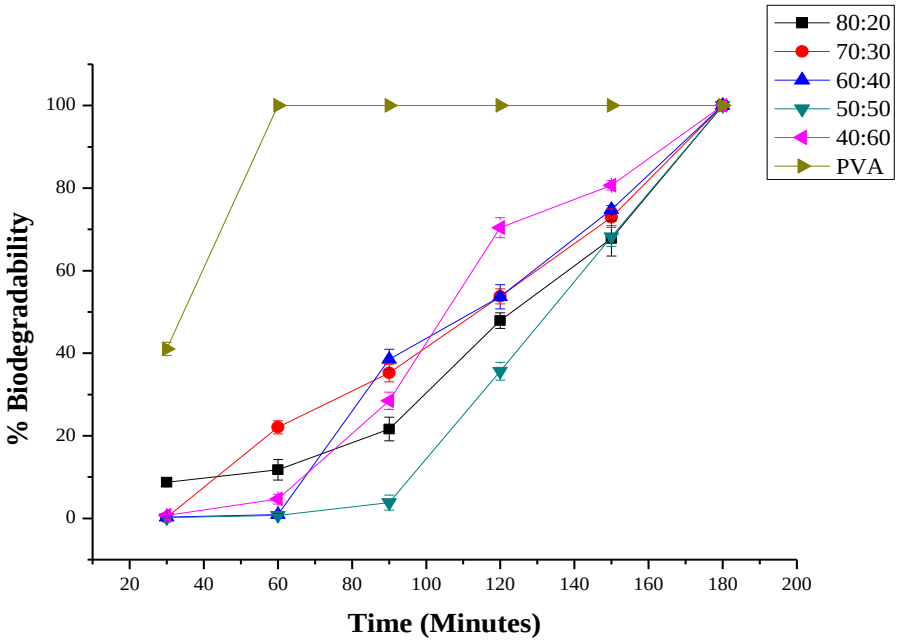

Fig. 10: Biodegradability analysis of polymeric blended fibers without crosslinking

5.9 Film Burst analysis

Film burst analysis was performed for six samples and the graph showed a bursting pattern (Fig. 11) which was then used to analyze the maximum Force (Rupture Strength) and mean distance at Break (Brittleness) which is shown in table 5.

Table 5: Values for rupture strength and mean distance of brittleness

S. No.	Samples (10% PVA + 2% Sodium Alginate)	Mean Max. Force 'Rupture Strength' (+/- S.D.) (g)	Mean Distance at Break 'Brittleness' (+/- S.D.) (mm)
1.	100:0	0.3630 ± 89	4.83 ± 20
2.	80:20	0.5221 ± 91	4.05 ± 57
3.	70:30	1.425 ± 56	4.97 ± 26
4.	60:40	1.625 ± 84	4.12 ± 83
5.	50:50	1.711 ± 35	5.27 ± 83
6.	40:60	1.534 ± 42	3.97 ± 87

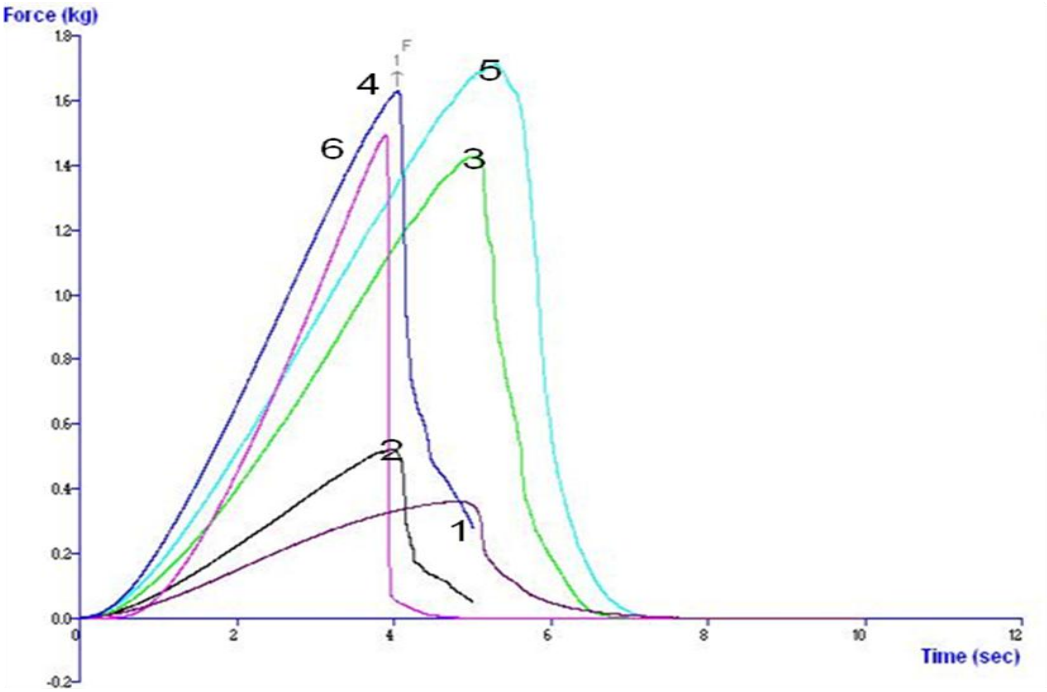

Fig. 11: Thin film burst analysis of PVA and Alginate blended fibers

5.10 Nanofiber fabrication with curcumin

Fibers were fabricated using the voltage of 72 kV, at a tip to collector distance of 12 cm and a rotation speed of 9.2 rpm. A mat was put on the collector electrode where the fibers were collected.

5.11 Results for characterization of curcumin loaded nanofibers

5.11.1 Scanning Electron Microscopy (SEM)

Fibers formed from the blended polymers of PVA and sodium alginate with and without drug were analyzed for their morphology viewing SEM images. The diameters of the fibers without drug were found in the range of 140-160 nm. Whereas loading of 5% curcumin had altered this range. Decrease in fiber diameter in the range of 120-130 nm was observed with the incorporation of curcumin. Both the fibers showed beads free morphology (Fig. 12).

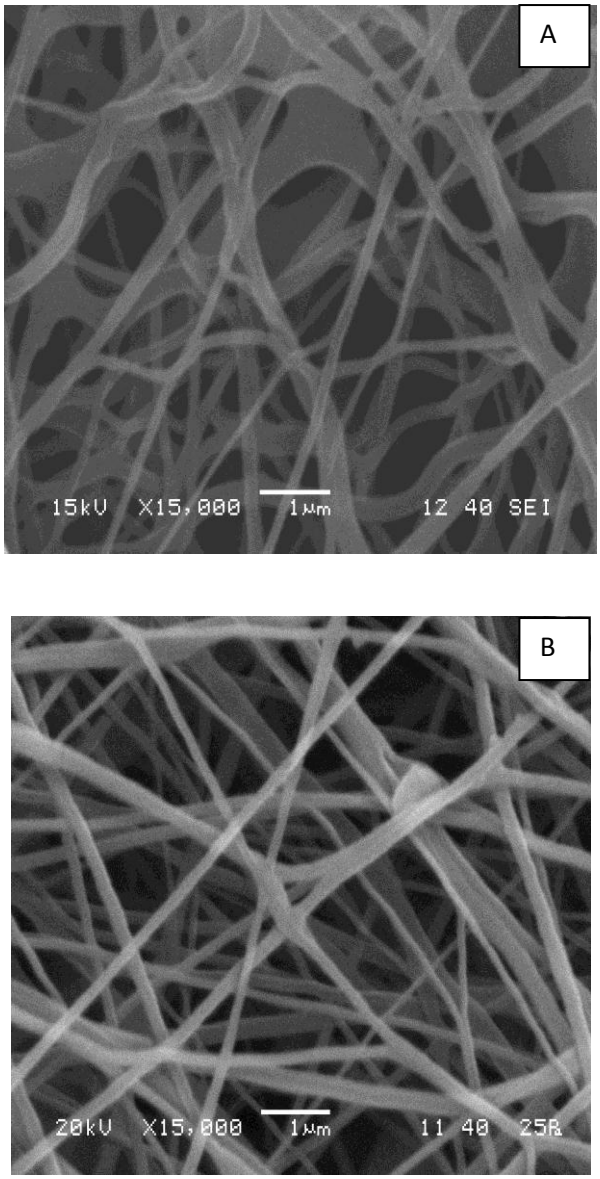

Fig. 12: SEM images depicting fiber morphologies of blened solutions of 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.11.2 X-Ray diffraction (XRD)

Presence of curcumin in the blended fibers of sodium alginate and PVA had shown a characteristic crystalline peak at a 2θ angle of 9.15º, 12.5º, 14.6º and 17.6º (Fig. 13). A peak at 19.3º was observed describing the hydrogen bonded interaction between PVA and sodium alginate in the sample without curcumin.

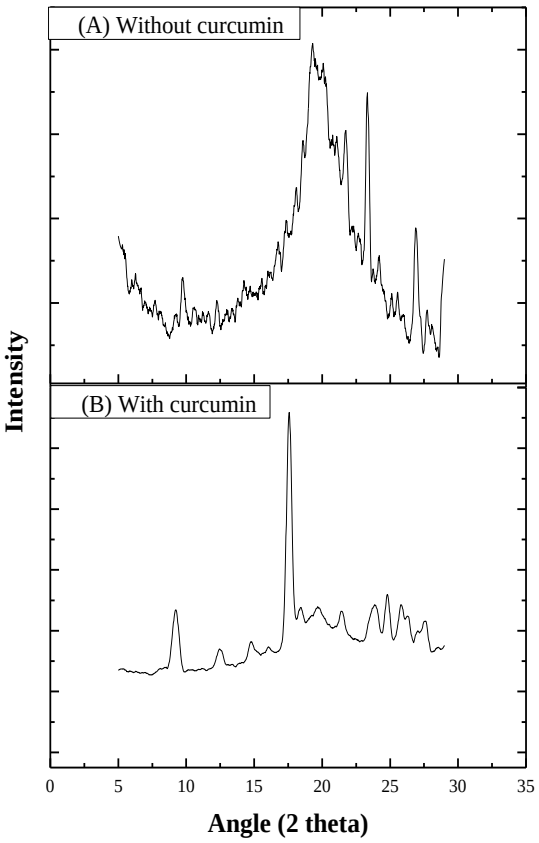

Fig. 13: XRD pattern for the fibers blended with blended 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.11.3 Fourier-transform infrared (FTIR) spectroscopy

Spectra obtained for FTIR analysis has showed a major peak at 3355 cm^{-1} for both the samples (blends with and without curcumin) indicating hydroxyl group (Fig. 13). Other common peaks were obtained at 2912 cm^{-1} and 2358 cm^{-1}. For the fibers containing curcumin, extra peaks were observed at 1638 cm^{-1} and 1510 cm^{-1} (as shown by Sahu et. al. 2010). Loading of curcumin did not show any significant alteration in the blended fibers of PVA and alginate.

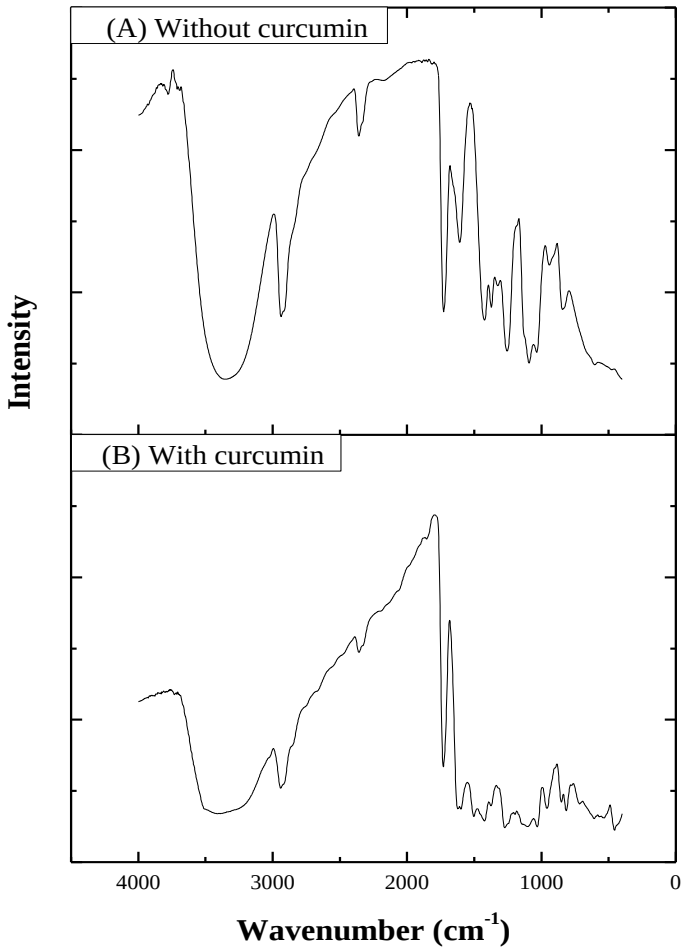

Fig. 14: FTIR analyis pattern for the fibers blended with blended 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.11.4 Differential scanning colorimetery (DSC)

Pattern observed for fibers with and without curcumin showed a shift in thermal denaturation peak. For sample containing equal amounts of PVA and sodium alginate without curcumin had shown the degradation peak at 190ºC which is found to shift at 176 ºC for the samples containing equal amounts of PVA and sodium alginate loaded with 5% curcumin (Fig. 15).

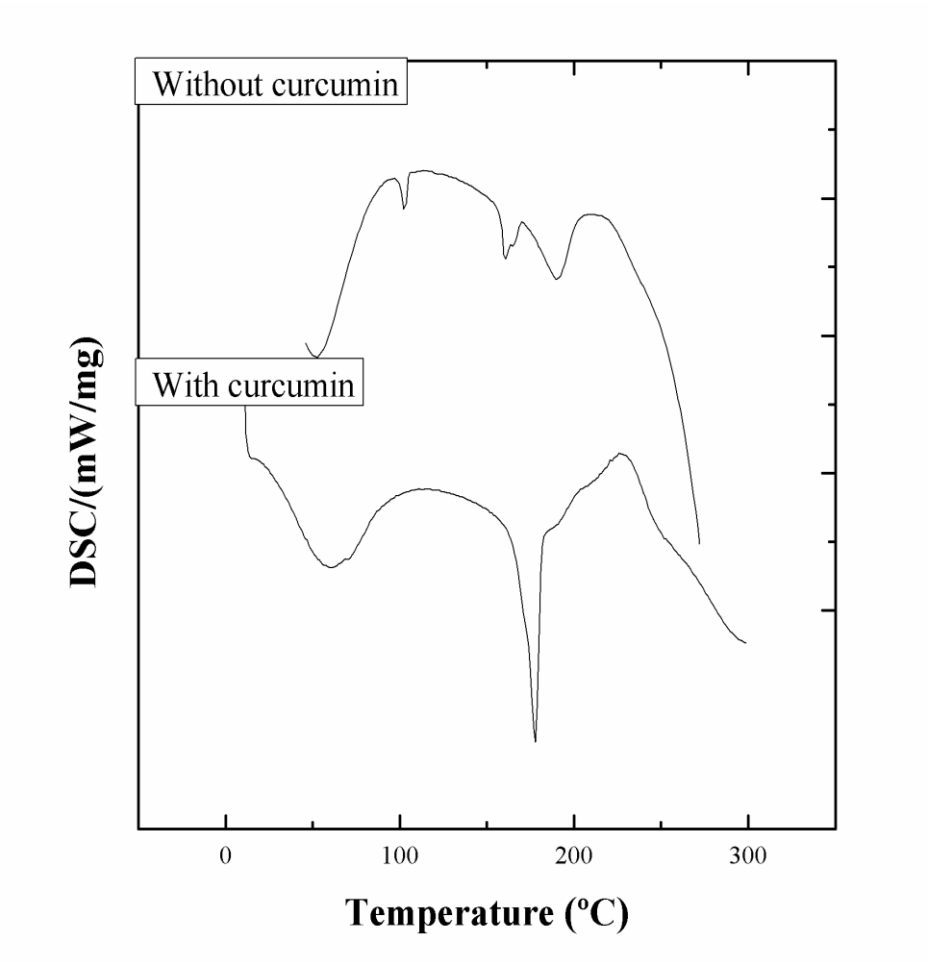

Fig. 15: DSC analyis pattern for the fibers blended with blended 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.11.5 Thermogravitometric Analysis (TGA)

Fibers with and without curcumin were analyzed for the pattern of their weight-loss using TGA for their degradation behaviour under a set of temperature. It was observed that the weight loss in the fibers had occurred at a temperature of around 200ºC (Fig. 16). With the finish of thermal decomposition, there were some residual matters found to be left behind by the fibers. Vinyl ester formation producing alkene and aldehyde groups is the major cause of such degradation.

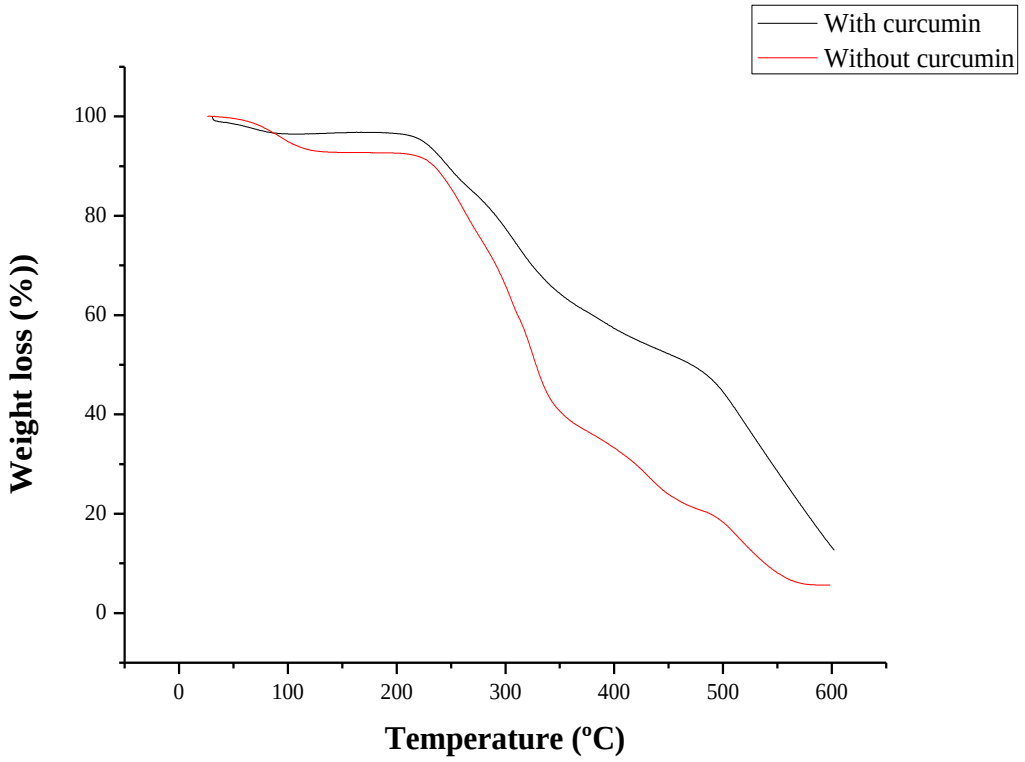

Fig. 16: TGA analyis pattern for the fibers blended with blended 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.12 Biodegradability Assay

Biodegradability percentage was calculated by incubating the samples in PBS at RT. The biodegradation behaviour of nanofibers prepared with PVA and PVA-Alginate with curcumin is shown in fig. 17. There would be loss of physiochemical properties of these polymers with the process of depolymerization. Degradation rate of blend of sodium alginate and PVA with curcumin was found to be much slower than that of sole PVA fibers. This slow rate of the degradation could be the result of higher cross linking between sodium alginate and glutaraldehyde making the fibers stable enough to resist the rate of depolymerization.

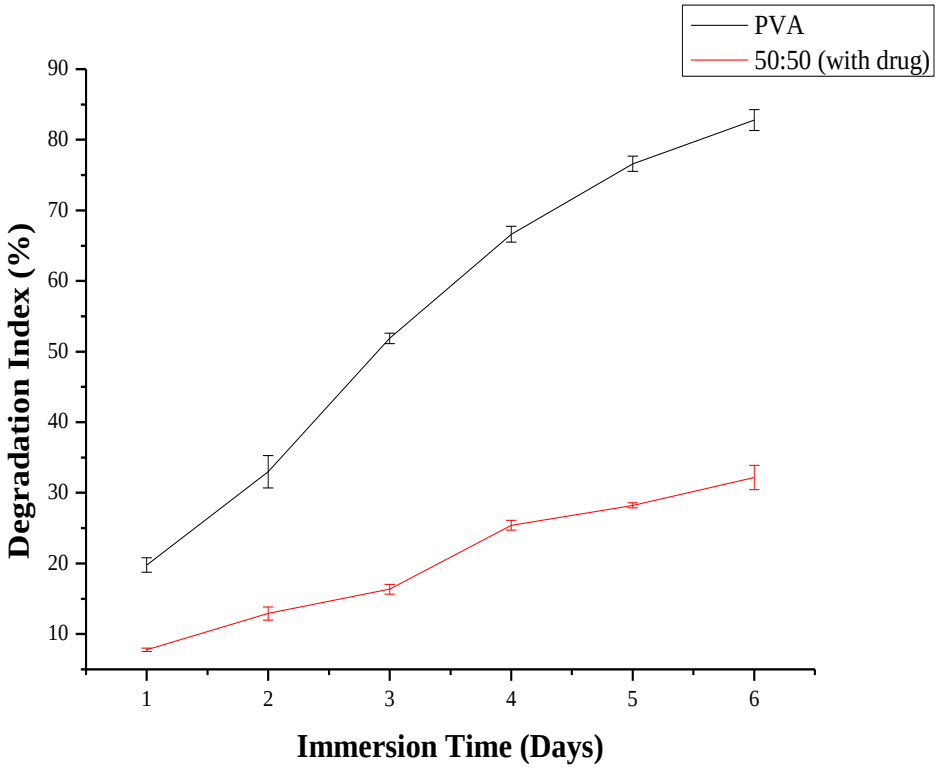

Fig. 17: Biodegradability analyis pattern for the fibers blended with blended 10% PVA and 2% Sodium alginate in ratio of 50:50. (A) without curcumin; (B) with 5% curcumin

5.13 Antimicrobial Activity

Antimicrobial activity of nanofiber matrix containing equal volumes of 10%PVA and 2% sodium alginate with 5% curcumin was checked. A matrix of equal volumes of 10% PVA and 2% sodium alginate without curcumin was used as a control. Fig. 18 describes the antimicrobial activity pattern. The zone of inhibition was calculated for the test sample which was found to be 15 mm whereas no such zone of inhibition was observed in the control sample.

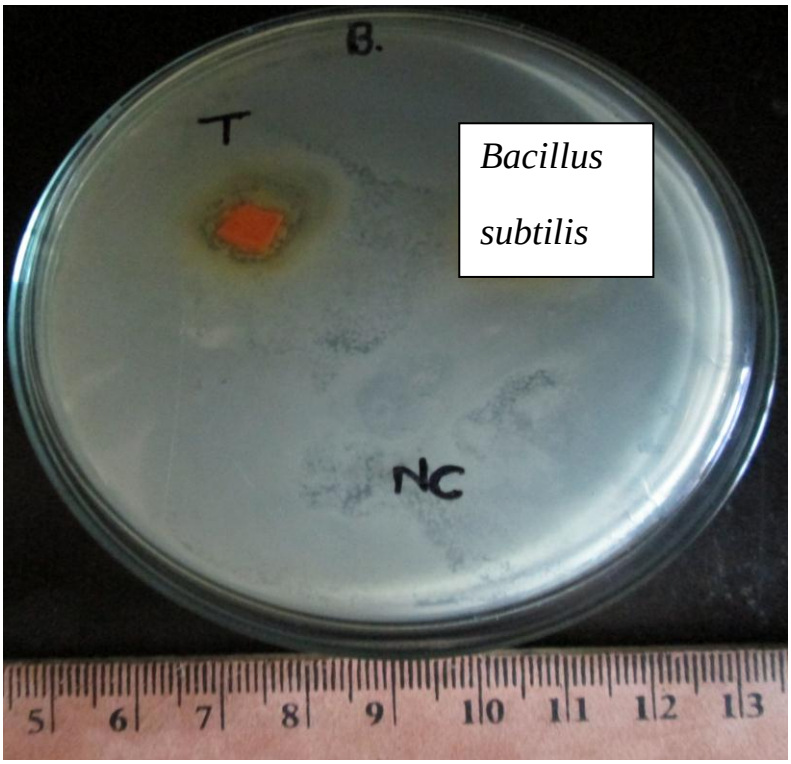

Fig. 18: Antimicrobial activity of curcumin loaded nanofiber matrix

5. 14 Release of curcumin *in vitro*

Nanofibers fabricated from equal amounts of alginate and PVA with and without curcumin were analyzed for their in vitro curcumin release. Media, sole curcumin and media samples with curcumin release were analyzed using fluorescence spectrophotometer at an excitation of 430 nm. Curcumin had shown a fluorescence peak at 560 nm whereas peak for DMEM media was observed at 490 nm. There was a shift of peak found in the samples containing media with curcumin release. The pure curcumin peak shifted from 560 nm to 590 nm for the samples those contained media with curcumin (Fig. 19).

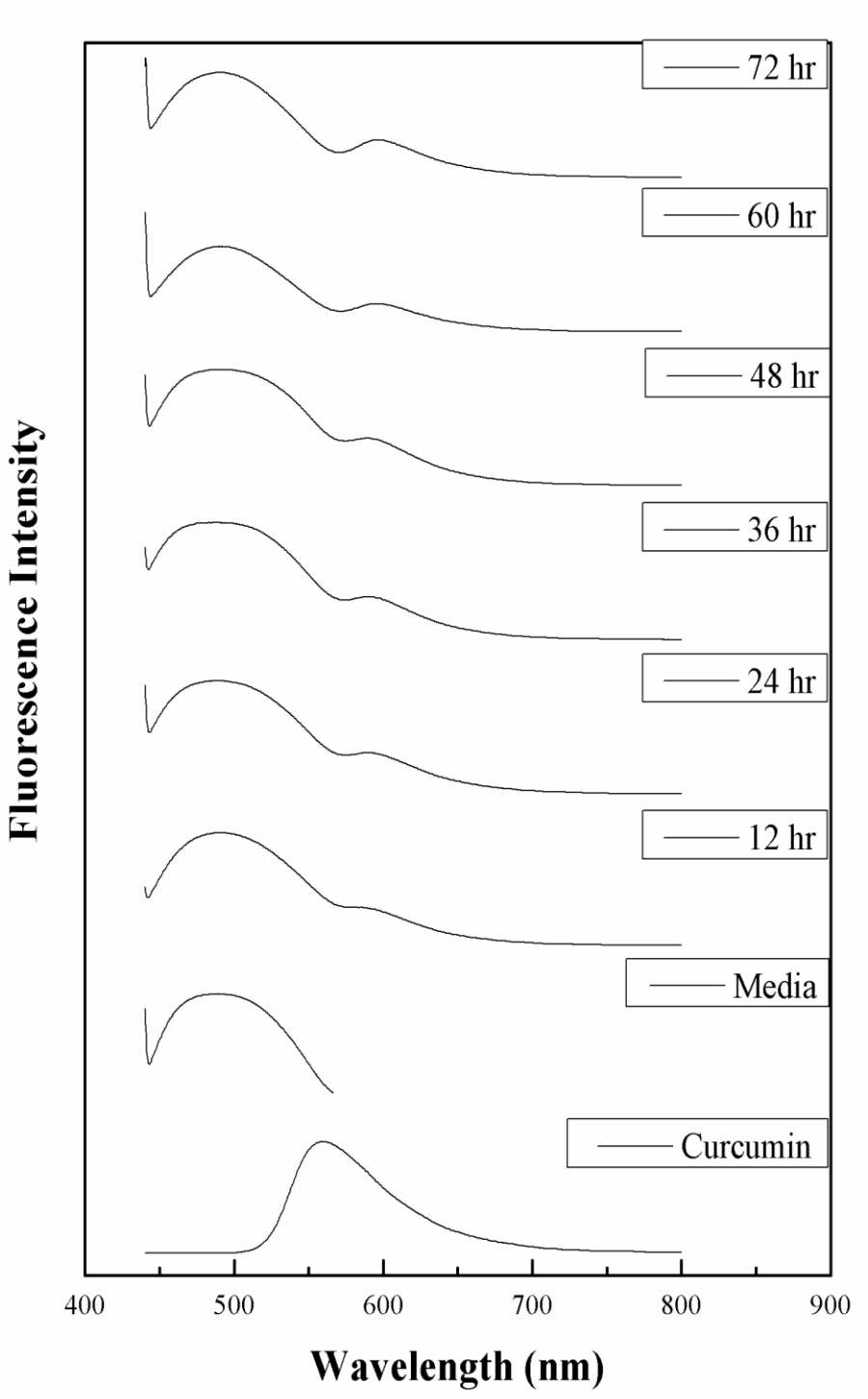

Fig. 19: In vitro curcumin release data

6. Conclusion

Polymeric blends of natural and synthetic polymers are preferred to fabricate nanofibrous scaffolds for tissue engineering to suffice mechanical properties, better processabilities, biocompatible and biodegradable nature and cost analysis. Nozzle less electrospinning has emerged out as an advanced technology in nanofiber fabrication at industrial mass scale. Pure alginate is not able to electrospun. To overcome this problem, synthetic polymers like PVA are required to blend with such natural polymers. Blending of PVA with sodium alginate improves the electospinnabilty by the formation of intermolecular hydrogen bonding and helps in the fabrication of uniform and continuous nanofibers. Blends of 2% sodium alginate and 10% PVA in different volumetric ratios were found to be the suitable combination for the fabrication of uniform nanofibers. Incorporation of higher amounts of sodium alginate was found to be helpful in reduction of fiber diameters. Curcumin loaded fibers were found to exhibit wound healing behaviour. Antimicrobial activity and drug release assay showed the measurable quality of fabricated fibers.

Optimized ratio of PVA and sodium alginate (50:50) was electrospun with 5% curcumin, a phytochemical; known for its wound healing, antioxidant and anti-inflammatory properties. Electrospun fibers have shown slow rate of biodegradability as compared to that of sole PVA fibers and thus can resist the rate of depolymerisation once implanted into body. Optimized results and fabricated scaffold can be used as an applicative measure for tissue engineering.

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